Antimicrobial Agents and Chemotherapy, Jan. 2011, p. 402–404 Vol. 55, No. 1
0066-4804/11/$12.00 doi:10.1128/AAC.01106-10

Prevalence and Mechanism of Resistance to Antimicrobial Agents in Group G Streptococcal Isolates from China[▽]

Jun Yin, Sangjie Yu, Xiaorong Liu, Ye Li, Wei Gao, Xiang Ma, and Yonghong Yang*

Department of Microbiology and Immunology, Beijing Children's Hospital Affiliated to Capital Medical University, Beijing 100045, China

Received 12 August 2010/Returned for modification 3 October 2010/Accepted 12 October 2010

Eighty group G streptococcal stains were collected from Chinese children. Susceptibility testing was done by a double-dilution and a disk diffusion method. PCR was used to test drug-resistant genes, and the χ^2 test and definite probability methods were used to test for statistically significant differences among the three groups. Thirty-four isolates (42.5%) showed resistance to erythromycin. There are differences between the resistance characteristics of group G streptococci from different regions of China.

Lancefield group G streptococci can cause pharyngitis and a variety of severe infections in humans (20). Besides being classified according to the Lancefield group carbohydrate, the beta-hemolytic streptococci are subdivided on the basis of whether they form large colonies or small colonies on sheep blood agar plates (8, 19, 20). The group G streptococcus (GGS) large-colony phenotypes are those usually associated with human infection.

Three different mechanisms of erythromycin resistance have been described (21). Target modification is mediated by an rRNA *erm* methylase that alters a site in the 23S rRNA common to the binding of macrolide, lincosamide, and streptogramin B antibiotics (21). Enzymes (EreA and EreB) that hydrolyze the lactone ring of the macrocyclic nucleus (2, 14) and phosphotransferases (type I [*mphA*] [10] and type II [11]) that inactivate macrolides by introducing a phosphate group on the 2′-hydroxyl group of the amino sugar have been reported in members of the family *Enterobacteriaceae* (18). The presence of multicomponent macrolide efflux pump systems in streptococci (*mefA*, *mefE*) (4, 18) has also been documented.

Eighty strains of GGS were collected from Chinese children <12 years old; 71 (42 from Guizhou Province, 3 from Beijing, and 26 from healthy children in Beijing) were identified to the species level as *Streptococcus dysgalactiae* subsp. *equisimilis* (large-colony phenotype), 8 (5 from Guizhou Province, 3 from Beijing) were *S. anginosus*, and the remaining isolate became nonviable before completion of the identification.

MICs of drugs were determined for all of the isolates using the Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, United Kingdom) supplemented with 5% sheep blood dilution method. All isolates were tested also against tetracycline and telithromycin by the disk diffusion method according to the performance standards of the Clinical and Laboratory Standards Institute (5). The microbiological and antibiotic susceptibility data are summarized in Table 1. The MICs of telithromycin were compatible with *S. pneumoniae*. Thirty-five isolates (43.8%) were resistant to erythromycin, 6 (12.5%) from Guizhou Province (MICs for 5 isolates, ≥32 μg/ml), 6 (100%) from patients in Beijing (MICs for 5 isolates, ≥32 μg/ml), and 23 (88.5%) from healthy children in Beijing (MICs for 22 isolates, ≥32 μg/ml). In addition, 2 (4.2%) isolates from Guizhou Province were resistant to telithromycin and erythromycin; 2 (33.3%) isolates from patients in Beijing and 9 (34.6%) isolates from healthy children in Beijing were resistant to telithromycin.

DNA was extracted as described previously and used for the detection of different macrolide resistance genes of GGS isolates by PCR (6). Primers for *ereA* (15), *ereB* (2), and *mphA* (10) were designed from published sequences to provide specific PCR products. Primers for the *mefA*/*E* genes were designed after comparison of the two sequences (4, 18).

We did not find any resistance genes carried by 46 sensitive isolates. Of the erythromycin-resistant isolates, 21 (61.8%) had the *ermB* gene, 20 (58.8%) had the *ermTR* gene, and 4 (11.8%) had the *mefA*/*E* gene. Both the *ermB* and *ermTR* genes were detected in 15 (44.1%) isolates, and 3 (8.8%) had both the *ermTR* and *mefA*/*E* genes. Of the 48 isolates from Guizhou Province, 2 (4.2%) had the *ermB* gene, 4 (8.3%) had the *ermTR* gene, 3 (6.3%) had the *mefA*/*E* gene, 2 (4.2%) had the *ermB* and *ermTR* genes, and 2 (4.2%) had both the *ermTR* and *mefA*/*E* genes. Of 6 isolates from patients in Beijing, 4 (66.7%) had the *ermB* gene, 2 (33.3%) had the *ermTR* gene, and only 1 (1.7%) had the *mefA*/*E* gene; of these isolates, 1 had both the *ermB* and *ermTR* genes (1.7%) and another isolate had both the *ermTR* and *mefA*/*E* genes (1.7%). Of the 26 isolates collected from healthy children in Beijing, 15 (57.7%) had the *ermB* gene, 13 (50.0%) had the *ermTR* gene, none had the *mefA*/*E* gene, and 12 (46.2%) had both the *ermB* and *ermTR* genes.

We used the χ^2 test to evaluate the relationship between resistance and PCR or definite probability methods when the χ^2 test was not appropriate. There were statistically significant differences ($P < 0.05$) among the three groups in resistance to erythromycin and clindamycin. The resistance characteristics of isolates from Guizhou Province and patients in Beijing showed significant differences ($P < 0.05$) for both erythromycin and clindamycin. The χ^2 test showed no significant difference among the three groups in the frequency of carriage of the *mefA*/*E* gene or both the *ermTR* and *mefA*/*E* genes.

* Corresponding author. Mailing address: Department of Microbiology and Immunology, Beijing Children's Hospital Affiliated to Capital Medical University, 56 Nan Lishi Road, Beijing 100045, China. Phone and fax: 008610 68029020. E-mail: yyh66@vip.sina.com.

▽ Published ahead of print on 18 October 2010.

TABLE 1. Susceptibilities of the GGS isolates described in this study

Drug[a]	No./% of isolates[b] from:								
	Guizhou Province			Patients in Beijing			Healthy children in Beijing		
	R	I	S	R	I	S	R	I	S
ERY	6/12.5	0/0	42/87.5	6/100	0/0	0/0	23/88.5	0/0	3/11.5
AZT	11/22.9	18/37.5	19/39.6	6/100	0/0	0/0	25/96.2	0/0	1/3.8
TEL	7/14.6	15/31.3	26/54.2	2/33.3	1/16.7	3/50.0	10/38.5	6/23.1	10/38.5
CLA	5/10.4	0/0	43/89.6	5/83.3	0/0	1/16.7	24/92.3	1/3.8	1/3.8
LVX	0/0	0/0	48/100	0/0	0/0	6/100	0/0	0/0	26/100
SMZ	0/0	3/6.3	45/100	0/0	0/0	6/100	0/0	0/0	26/100
TER	39/81.3	3/6.3	6/12.5	4/66.7	0/0	2/33.3	21/80.8	3/11.5	2/7.7

[a] ERY, erythromycin; AZT, azithromycin; TEL, telithromycin; CLA, clarithromycin; LVX, levofloxacin; SMZ, trimethoprim-sulfamethoxazole; TER, tetracycline.
[b] R, resistant; I, intermediate; S, susceptible.

There has been no detailed report of drug-resistant GGS in China. In view of the increasing frequency of GGS drug resistance worldwide, it is important that studies be conducted and reported regularly. This study showed that the rates of resistance to other antimicrobial agents among our 80 isolates were variable: the resistance of Beijing isolates, especially to macrolides, was much higher than that of the Guizhou Province isolates. Different patterns of antimicrobial usage, which lead to variable selective pressure on resistance, might be one of the primary factors (13). The prevalence of erythromycin resistance in GGS is reported as 33.3% in Spain (12) and 3.5% in Finland (9), which is higher than that in Guizhou Province (12.5%) but much lower than that in Beijing (100% of the isolates from patients and 88.5% of those from healthy people). Other factors might include the distribution of specific serotypes and the spread of resistant clones within certain regions. These high rates of macrolide resistance probably reflect the high level of antibiotic usage in China. Our studies of multiple centers in China have shown that the consumption of macrolides was very high, more than half of the total defined daily dose volume (22).

Telithromycin is not allowed to be used in China, but resistance to telithromycin was found in this study. Telithromycin is the first antibiotic belonging to a new class of 14-membered ring macrolides named ketolides. Ketolides belong to the macrolide-lincosamide-streptogramin B group that has been developed for the treatment of upper and lower respiratory tract infections caused by common and atypical pathogens, including resistant streptococci (1). Compared with macrolides, the molecular structure of telithromycin is characterized by the absence of the cladinose group, which is probably linked to the induction of resistance to macrolides (3).

In this study, the characteristic of erythromycin resistance of GGS had a close relationship with the geographic region of occurrence. Analysis showed a significant difference between isolates from Guizhou Province and isolates from Beijing. There was no significant difference between isolates from patients and those from healthy subjects in Beijing. Perhaps the invasiveness factor has a closer relationship with the region of occurrence than it has with resistance. Although beta-hemolytic streptococci remain widely susceptible to penicillin, resistance to macrolides is rather common. It is well known that beta-hemolytic streptococcal resistance can spread across countries and even continents. Hence, it is important that the susceptibility patterns of GGS from various regions of China be recorded on a regular basis so that trends can be monitored closely and treatment guidelines can be modified as appropriate.

There are major geographic differences associated with the mechanisms of macrolide resistance. The *mefA* gene was first identified in group A streptococci (17). In one study, it was noted that 31 (97%) of 32 erythromycin-resistant beta-hemolytic GGS isolates recovered in Finland possessed *erm* genes, of which 30 (94%) possessed the *ermTR* gene and only 1 possessed the *ermB* gene (9). In the present study, 4 (11.8%) of 34 GGS isolates with resistance to erythromycin possessed the *mef* gene, 25 (61.8%) of 34 possessed the *ermB* gene, and 20 (58.8%) of 34 possessed the *ermTR* gene. The molecular epidemiology of GGS in China showed many differences from earlier reports.

We conclude that classical antimicrobial agents used to treat GGS infections have good activity against clinically significant isolates, but the presence of macrolide resistance suggests that there should be careful surveillance of isolates. Furthermore, the resistance characteristic of GGS isolates vary between regions of China and the resistance mechanisms show differences from those found in other countries.

REFERENCES

1. **Ackermann, G., and A. C. Rodloff.** 2003. Drugs of the 21st century: telithromycin (HMR 3647)—the first ketolide. J. Antimicrob. Chemother. **51:**497–511.
2. **Arthur, M., D. Autissier, and P. Courvalin.** 1986. Analysis of the nucleotide sequence of the ereB gene encoding the erythromycin esterase type II. Nucleic Acids Res. **14:**4987–4999.
3. **Bonnefoy, A., A. M. Girard, C. Agouridas, and J. F. Chantot.** 1997. Ketolides lack inducibility properties of MLS(B) resistance phenotype. J. Antimicrob. Chemother. **40:**85–90.
4. **Clancy, J., J. Petitpas, F. Dib-Hajj, W. Yuan, M. Cronan, A. V. Kamath, J. Bergeron, and J. A. Retsema.** 1996. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, mefA, from *Streptococcus pyogenes*. Mol. Microbiol. **22:**867–879.
5. **Clinical and Laboratory Standards Institute.** 2008. Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement. CLSI/NCCLS document M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.
6. **Eady, E. A., J. I. Ross, J. L. Tipper, C. E. Walters, J. H. Cove, and W. C. Noble.** 1993. Distribution of genes encoding erythromycin ribosomal methylases and an erythromycin efflux pump in epidemiologically distinct groups of staphylococci. J. Antimicrob. Chemother. **31:**211–217.
7. **Hill, H. R., G. G. Caldwell, E. Wilson, D. Hager, and R. A. Zimmerman.** 1969. Epidemic of pharyngitis due to streptococci of Lancefield group G. Lancet **(ii):**371–374.
8. **Kaplan, E. L., and M. A. Gerber,** 1997. Group A, group C, and group G beta-hemolytic streptococcal infections, p. 1084–1088. *In* R. D. Felgin and

T. D. Cherry (ed.), Textbook of pediatric infectious diseases, 4th ed. W. B. Saunders Company, Philadelphia, PA.

9. **Kataja, J., H. Seppälä, M. Skurnik, H. Sarkkinen, and P. Huovinen.** 1998. Different erythromycin resistance mechanisms in group C and group G streptococci. Antimicrob. Agents Chemother. **42:**1493–1494.
10. **Kim, S. K., M. C. Baek, S. S. Choi, B. K. Kim, and E. C. Choi.** 1996. Nucleotide sequence, expression and transcriptional analysis of the *Escherichia coli mphK* gene encoding macrolide-phosphotransferase K. Molecules Cells **6:**153–160.
11. **Kono, M., K. O'Hara, and T. Ebisu.** 1992. Purification and characterization of macrolide 2′-phosphotransferase type II from a strain of *Escherichia coli* highly resistant to macrolide antibiotics. FEMS. Microbiol. Lett. **76:**89–94.
12. **Merino Díaz, L., M. J. Torres Sánchez, and J. Aznar Martín.** 2008. Prevalence and mechanisms of erythromycin and clindamycin resistance in clinical isolates of beta-haemolytic streptococci of Lancefield groups A, B, C and G in Seville, Spain. Clin. Microbiol. Infect. **14:**85–87.
13. **Negri, M. C., M. I. Morosini, E. Loza, and F. Baquero.** 1994. In vitro selective antibiotic concentrations of beta-lactams for penicillin-resistant *Streptococcus pneumoniae* populations. Antimicrob. Agents Chemother. **38:** 122–125.
14. **O'Hara, K.** 1994. Application of nuclear magnetic resonance spectrometry to measure the activity of bacterial macrolide esterase. Microbios **79:**231–239.
15. **Ounissi, H., and P. Courvalin.** 1985. Nucleotide sequence of the gene ereA encoding the erythromycin esterase in *Escherichia coli*. Gene **35:**271–278.
16. **Ross, J. I., E. A. Eady, J. H. Cove, and S. Baumberg.** 1995. Identification of a chromosomally encoded ABC-transport system with which the staphylococcal erythromycin exporter MsrA may interact. Gene **153:**93–98.
17. **Sutcliffe, J., T. Grebe, A. Tait-Kamradt, and L. Wondrack.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:**2562–2566.
18. **Tait-Kamradt, A., J. Clancy, M. Cronan, F. Dib-Hajj, L. Wondrack, W. Yuan, and J. Sutcliffe.** 1997. *mefE* is necessary for the erythromycin-resistant M phenotype in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **41:**2251–2255.
19. **Vandamme, P., B. Pot, E. Falsen, K. Kersters, and L. A. Devriese.** 1996. Taxonomic study of Lancefield streptococcal groups C, G, and L (*Streptococcus dysgalactiae*) and proposal of *S. dysgalactiae* subsp. *equisimilis* subsp. nov. Int. J. Syst. Bacteriol. **46:**774–781.
20. **Vartian, C., P. I. Lerner, D. M. Shlaes, and K. V. Gopalakrishna.** 1985. Infections due to Lancefield group G streptococci. Medicine (Baltimore) **64:**75–88.
21. **Weisblum, B.** 1995. Erythromycin resistance by ribosome modification. Antimicrob. Agents Chemother. **39:**577–585.
22. **Zhang, W., X. Shen, Y. Wang, Y. Chen, M. Huang, Q. Zeng, M. Fan, U. Bergman, and Y. Yang.** 2008. Outpatient antibiotic use and assessment of antibiotic guidelines in Chinese children's hospitals. Eur. J. Clin. Pharmacol. **64:**821–828.